Madroño, Vol. 61, No. 1, pp. 9–15, 2014

CHROMOSOME NUMBER AND REPRODUCTIVE ATTRIBUTES FOR *ERIGERON LEMMONII* (ASTERACEAE), A CLIFF-DWELLING ENDEMIC OF SOUTHEASTERN ARIZONA

Richard D. Noyes
Department of Biology, University of Central Arkansas, Conway, AR 72035
rnoyes@uca.edu

Pamela Bailey
U.S. Army Engineer Research and Development Center, Vicksburg, MS 39180

Abstract

Erigeron lemmonii A. Gray, restricted to Scheelite Canyon in the Huachuca Range, Arizona, has previously been proposed for federal listing as an endangered species but basic cytological and reproductive information has been wanting. The first chromosome count for the species is $2n = 18$, which is the common diploid number in *Erigeron*. Analyses of eight plants from five disparate sites within the population show that pollen averages 85.8% staining in cotton blue in lactophenol. Microscopic observation using differential interference contrast optics shows that *E. lemmonii* combines about equally monosporic and bisporic megagametophyte development within a single capitulum. Despite variability in developmental route, the egg apparatus among mature megagametophytes appears to be nearly uniform in structure. In greenhouse culture, isolated plants fail to set seed indicating that plants are probably self-incompatible. Controlled crosses yield seed, but variation in seed set intimates the possible presence of genetic barriers within the population.

Key Words: bispory, conservation, endemic, *Erigeron*, *Erigeron lemmonii*, gametogenesis, megasporogenesis, monospory.

Erigeron L. (Asteraceae) consists of about 390 species with 173 species documented for North America north of México (Nesom 2006). In the United States, most species occur in the montane and arid West. General morphological uniformity can make determining the identity of species difficult; most of the taxa are low perennials with simple or lobed, alternate, one-nerved leaves, and white to light-purple rays and yellow discs. Cronquist (1947) even concluded that indument was the most reliable character for species delimitation. Further complicating systematic elucidation in *Erigeron* is the occurrence of apomictic complexes that include local polyploid hybrid populations (microspecies) that reproduce asexually by seed. Despite these issues, a systematic framework *Erigeron* is maturing based on a combination of morphological and molecular analyses (Noyes 2000; Nesom 2008).

It is common for *Erigeron* species to be locally endemic and known from relatively few populations in specialized habitats. For instance, in Arizona, out of 42 described *Erigeron* species, 13 have global conservation status ranks (www.NatureServe.org/explorer) of G1 (four taxa; critically imperiled), G2 (six taxa; imperiled), or G3 (three taxa; vulnerable). To effectively manage such restricted and sensitive plant species, basic biosystematic data are essential. Such data may include assessments of chromosome number, breeding system (selfing vs. self-incompatible), mode of reproduction (apomictic vs. sexual), and phylogenetic relationship. For many *Erigeron* species, these data are lacking.

Erigeron lemmonii A. Gray is known only from Scheelite Canyon in the Huachuca Mountains of Cochise County, Arizona. It was described in 1883 based on a collection by John Gill Lemmon made the previous year (Gray 1883). It is classified as a member of *Erigeron* sect. *Oligotrichium* Nutt. (Nesom 2008) and is a decumbent-ascending perennial forming clumps in crevices and on ledges of vertical limestone cliffs within the canyon. Plants produce relatively long, arching stems that give rise to solitary (or few) capitula on ascending branches. *Erigeron lemmonii* has a global rank of G1 (www.natureserve.org/explorer). Based in part on a report indicating that it was known from only 108 individuals (Gori et al. 1990), it was proposed as a candidate for protection under the Endangered Species Act in 1993. Subsequent extensive census of suitable habitat in the Huachuca Mountains did not uncover new populations, but additional plants discovered within Scheelite Canyon brought the estimated total number of individuals to about 950 (Malusa 2006). In consideration of these new data and in determining that *E. lemmonii* was stable and unlikely to be extirpated, it was removed from the candidate list (U.S. Department of the Interior, Fish and Wildlife Service 2012).

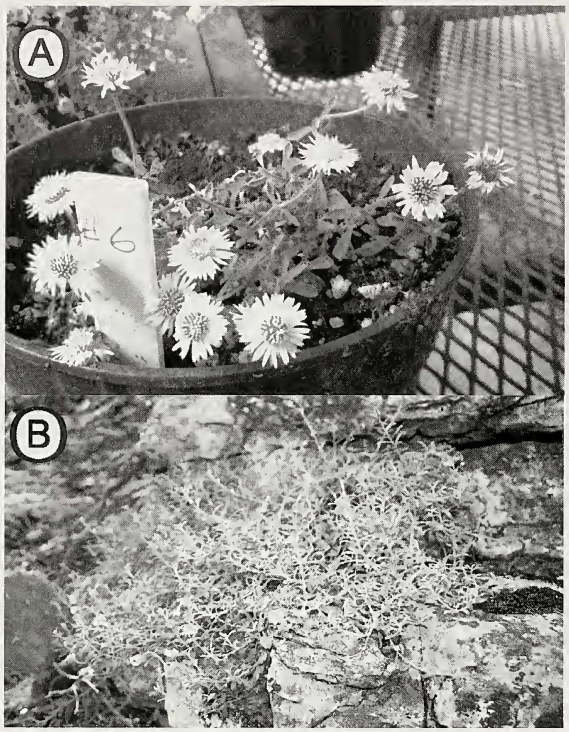

FIG. 1. Photographs of *Erigeron lemmonii*. A. In greenhouse culture at the University of Central Arkansas from rooted ramets collected in the field. B. On a cliff ledge in Scheelite Canyon, AZ. Capitula are approximately one cm diam. Distal leaves along flowering branches are typically entire (as viewed in A); more basally disposed leaves are commonly three- to five-lobed.

MATERIALS AND METHODS

In the early June 2012, vegetative branches from nine plants of *Erigeron lemmonii* (Fig. 1) were collected and sent to the University of Central Arkansas, Conway. Plants were sampled from four sites in Scheelite Canyon: North Main Face, sample #1; Main Face, samples #2–6; Boulder, sample #7; Owl Canyon, samples #8–9 (Fig. 2). Upon arrival, bases of the stems were dipped in rooting hormone powder (Green Light Organic Rooting Hormone, Green Light Co., San Antonio, TX, greenlightco.com), placed in Fafard Professional Potting Mix (Conrad Fafard Inc., Agawam, MA, www.fafard.com/Products/) in four-inch pots, and provided with natural lighting and moderate watering regimen. As the plants came into flower in the greenhouse, their ability to make seed autonomously was assessed by inspecting shattered mature heads under a dissecting microscope using transmitted illumination. In *Erigeron*, filled cypselae are opaque and light brown; empty ones are transparent. Herbarium vouchers of the North Main Face specimen (RDN #1687) were prepared and deposited at UCAC and ARIZ.

Chromosome number and pollen stainability were determined for all plants. Root-tips were collected in the early morning and pretreated in 8-hydroxyquinoline for four hours and then fixed in 3:1 ethanol: acetic acid. Root-tips were then digested in 15% (~2 mol/L) HCl for 26 minutes at room temperature, rinsed in distilled water, macerated, stained with acetocarmine, squashed under a cover slip, and viewed at 1000× using bright-field microscopy. To assess stainability, newly shed pollen was stained in Cotton Blue in lactophenol for four days and evaluated with bright field microscopy at 400× (Stanley and Linskens 1974). Stainability was scored as percentage of darkly staining grains in a sample of 300 grains. Pollen size was estimated for the Main Face-4 accession by measuring on digital images a sample of 85 grains with diameters estimated from average mid-exine to mid-exine points.

Reproductive development was evaluated for the North Main Face plant. Capitula at three stages of development were studied: stage one – pre-anthesis, to observe initial division of the megasporocyte; stage two – early anthesis, to view condition of mature female gametophytes; stage three – three days post anthesis, to detect evidence for autonomous embryo or endosperm formation. Capitula were fixed in FAA for two weeks and then dehydrated in 100% ethanol and cleared in methyl salicylate (Herr 1971). Ovaries (each bearing a single ovule) were dissected from the cleared florets and arrayed under a cover slip held in place with rubber cement. Cellular detail of ovules was observed at 600× using differential interference contrast optics (D.I.C).

All microscopic observations were performed using an Olympus B54 microscope. Images were made with a CCD 8-bit digital camera, and measurements were made using AnalySIS (version 3.1) image-capturing software (Soft Imaging System, GmbH 1989–2001).

The ability of plants to make seed by outcrossing was evaluated with controlled crosses among the plants in greenhouse culture. Four inter-site crosses were performed. Pollinations were made by removing a capitulum at anthesis from the pollen donor and thoroughly brushing it against newly emerging stigmas of the seed parent. Pollinations for a single cross were performed over five days to ensure pollen transfer to all florets as they opened in the capitulum of the seed parent. All florets of a capitulum opened within five days.

RESULTS AND DISCUSSION

All nine ramets of *Erigeron lemmonii* that had been treated with rooting hormone developed nodal roots after about four weeks and developed aerial branches and flowers after about 10 weeks

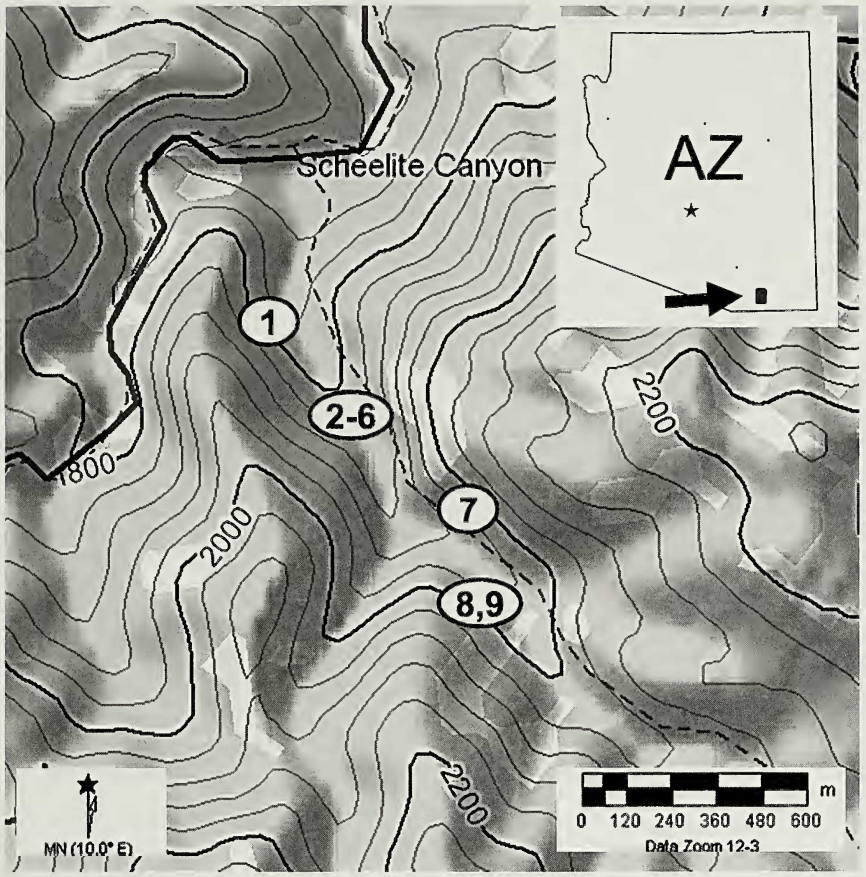

FIG. 2. Map of Scheelite Canyon, Huachuca Mountains, Arizona, showing location of plants collected for study. 1: North Main Face; 2–6: Main Face; 7: Boulder; 8–9: Owl Canyon.

(Fig. 1). Subsequent tests documented that ramets in standard soil without rooting hormone would stay green and appear healthy but would not develop roots. Ramets treated with rooting hormone but placed in potting soil-sand mixtures also rooted but more slowly than in potting soil alone. All rooted plants thrived in the greenhouses of the University of Central Arkansas and did not require special watering or light treatment. Plants flowered in successive flushes about every three months with flowers emerging on new branches produced from near the base of the plant. Mature capitula inspected for each plant always consisted of empty ovaries; no filled cypselae were ever observed. We conclude that despite the restricted distribution and habitat of the species, *E. lemmonii* is easily cultured in greenhouse conditions and is not capable of making seed autonomously either by selfing or apomixis. The evidence is consistent with the hypothesis that *E. lemmonii* possesses sporophytic incompatibility, as has been described for other Asteraceae (Gerstel 1950; de Nettancourt 1977).

Chromosome counts for all nine plants revealed $2n = 18$, the first count for the species (Table 1, Fig. 3A). This is the common diploid number for *Erigeron* and the presumed ancestral number for tribe Astereae (Brouillet et al. 2009). The chromosome complement is nearly uniform, consisting of approximately equal-length chromosomes about three μm long. B-chromosomes were not observed. Pollen produced by the eight plants had high percentage of staining grains (mean 85.8%; Table 1; Fig. 3B). The average measure of the diameters of 85 grains for the Main Face-4 sample was 16.5 μm (SD = 0.79) and the grains all had three evident pores. This grain size is only modestly greater than the mean value of 14.9 μm obtained for 36 diploid populations of *E. strigosus* Muhl. ex Willd. of eastern North America (Noyes and Allison 2005).

For the capitula of North Main Face *Erigeron lemmonii*, we observed that each ovary consisted of a single standard unitegmic, tenuinucellate, anatropous ovule bearing a single megasporocyte (Fig. 4A). This condition is typical for *Erigeron*, though it has been reported that some species have multiple megasporocytes within a common nucellus that then compete for dominance in subsequent development (Harling 1951). We

TABLE 1. CHROMOSOME NUMBERS AND POLLEN STAINABILITY FOR *ERIGERON LEMMONII*, SCHEELITE CANYON, AZ. Chromosome numbers determined from acetocarmine squashes of root-tips. Pollen stainability reported as percentage of grains (out of 300) darkly and uniformly staining in cotton blue in lactophenol.

Plant ID	Chromosomes (*2n*)	Pollen stained (%)
1. North Main Face	18	95.3
2. Main Face - 1	18	87.3
3. Main Face - 2	18	76.7
4. Main Face - 3	18	92.7
5. Main Face - 4	18	86.7
6. Main Face - 5	18	80.7
7. Boulder	18	71.7
8. Owl Canyon - 1	18	96
9. Owl Canyon - 2	18	84.7
Mean (SD)		85.8 (8.3)

observed variation in the number and placement of cell walls separating the four products of meiosis. Five patterns were observed, three of which were approximately equal in frequency. Of 130 ovules, 42 (32.3%) were consistent with typical monosporic development, exhibiting four nuclei partitioned into separate spores by cell walls (Fig. 4B), and 46 (35.4%) exhibited a bisporic pattern with two cells each bearing two nuclei (Fig. 4C), which results when cell walls form between the two products of meiosis I but no walls form between the products of meiosis II. The third common type (33 ovules, 25.4%) appeared to be a blend of tetrasporic and bisporic development yielding three cells; the micropylar cell contained two nuclei and the two distal cells each contained a single nucleus (Fig. 4D). This pattern evidently results when a cell wall is deposited following meiosis I, but following meiosis II a wall is formed only between nuclei in the chalazal cell. The two low-frequency patterns observed are also interpreted to be developmental mixtures: a three-celled type (8 ovules, 6.2%) similar to the third common type above except that the two nucleate cell was chalazal rather than micropylar, and a type was observed only once (0.8%) that consisted of two cells, a uninucleate micropylar cell and a trinucleate chalazal cell. Tetraspory, i.e., the formation of a single coenospore containing all four products of meiosis, was not observed. In subsequent development, we observed most commonly (46 of 59 observations, 78.0%) the expansion and vacuolization of the chalazal spore (whether one- or two-nucleate) and compression and ultimate degeneration of the micropylar spore(s) (Fig. 4E, F, G). In the other 13 ovules (22.0%), we observed expansion of the micropylar or a median spore (Fig. 4H). In sum, early reproductive development for *E. lemmonii* is characterized by an equal mixture of mono- and bisporic types within a single capitulum and a diversity of intermediate forms.

The mature megagametophytes yielded egg apparati that were highly regular in structure. Of 124 gametophytes observed, 108 (87.1%) consisted of a single domed egg cell, two wedge shaped synergids forming the micropylar terminus of the gametophyte, and two polar nuclei within the central cell usually directly adjacent to the egg cell (Fig. 4I). Of the remaining ovules observed, ten (8.1%) either apparently lacked a gametophyte, or the gametophyte was collapsed into a dense mass. In six ovules (4.8%), gametophytic cells did not yield a recognizable egg apparatus. For the regular megagametophytes, the polar nuclei were fused into a common fusion nucleus and the degree of fusion varied. Of 62 ovules, 47 (75.8%) possessed a single fusion nucleus with a single large nucleolus, ten ovules

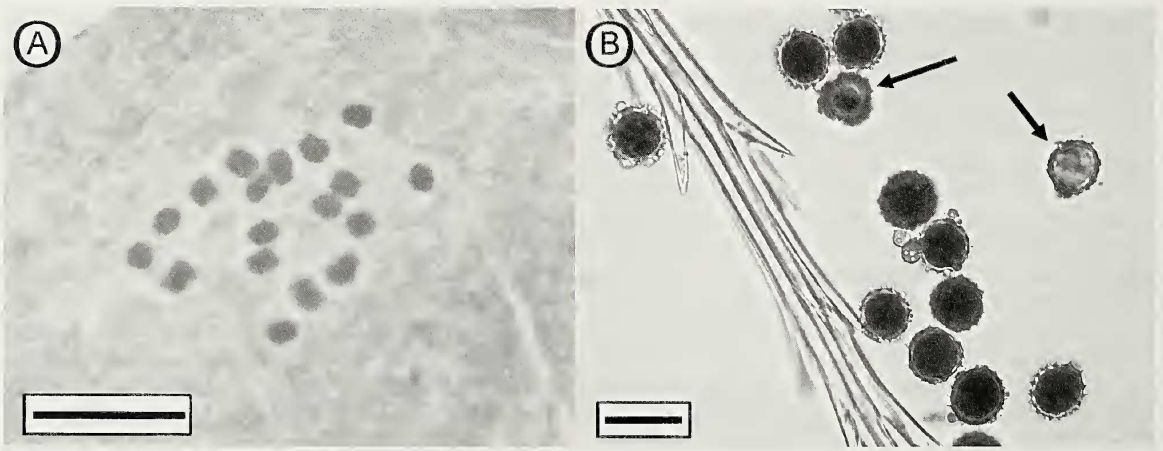

FIG. 3. Chromosome complement and pollen for *Erigeron lemmonii*. A. Acetocarmine chromosome squash for Main Face-5 showing $2n = 18$. B. Putatively viable pollen stained with cotton blue in lactophenol is dark; lightly and irregularly stained grains (arrows) are likely inviable. Scale bars = 20 μm.

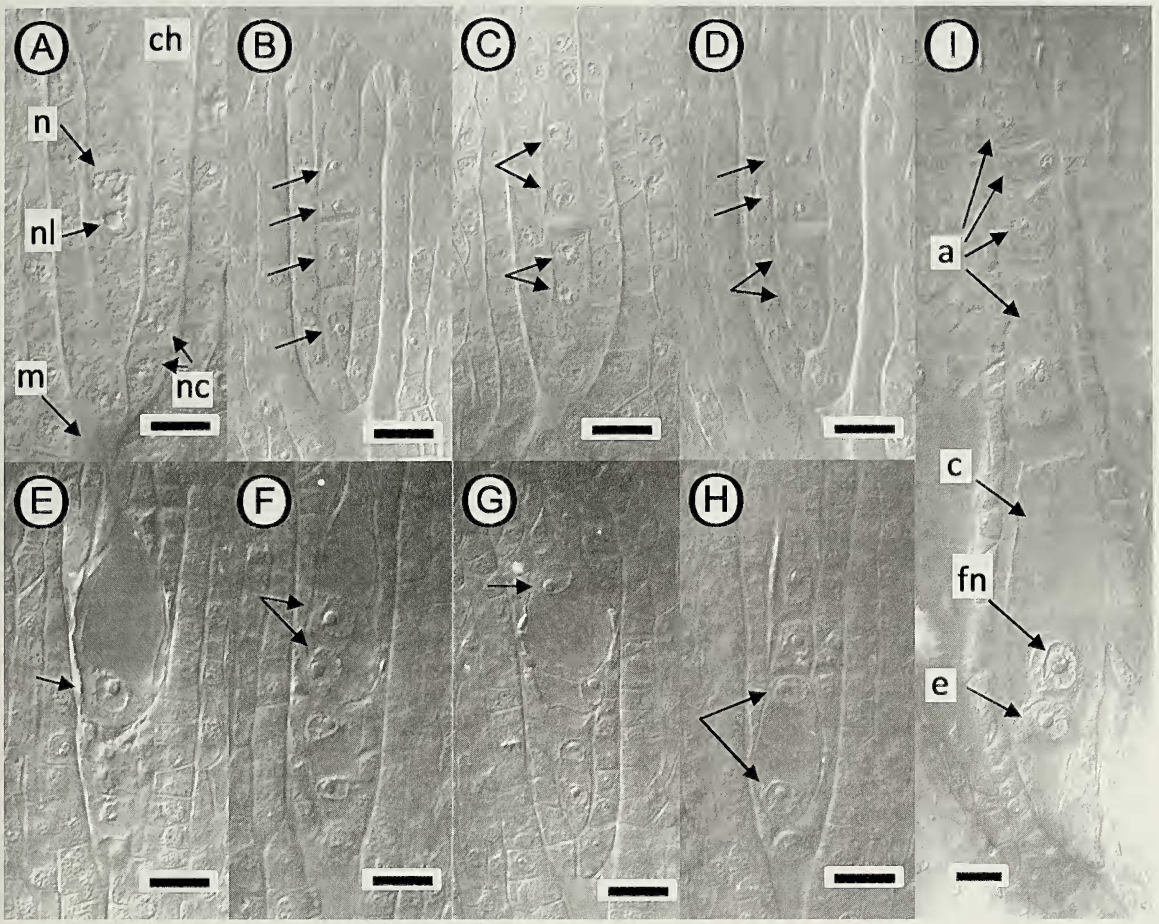

FIG. 4. Megagametophyte development for North Main Face *Erigeron lemmonii*. Abbreviations; a = antipodal cells, c = central cell, ch = chalazal region, e = egg cell, fn = fusion nucleus, m = micropyle, n = nucleus, nc = nucellus, nl = nucleolus. A. Megasporocyte within the ovule prior to meiotic division. B–D. Alternative spore arrangements resulting from meiosis. B. Four uninucleate megaspores indicative of monospory. C. Two binucleate megaspores indicative of bispory. D. Two uninucleate and one binucleate megaspore indicative of megasporogenesis intermediate between monospory and bispory. E–H. Early megaspore germination patterns prior to the first mitotic division. Arrows indicate nuclei within the selected developing spore. E. Enlargement of uninucleate chalazal spore, with compression of three micropylar spores. F. Enlargement of a binucleate chalazal spore, with compression of a micropylar binucleate spore. G. Enlargement of a uninucleate chalazal spore, with compression of micropylar uninucleate and binucleate spores. H. Enlargement of a micropylar binucleate spore, with early compression of two uninucleate chalazal spores. I. Mature megagametophyte showing egg, fusion nucleus, and antipodal cells and nuclei within the chalazal panhandle; synergids present but not visible in this view. Scale bars = 20 μm.

(16.1%) had a fusion nucleus that was divided by a nuclear membrane into two compartments corresponding to the two polar nuclei, and five ovules (8.1%) contained a single fusion nucleus with two, distinct nucleoli.

In contrast to the egg apparatus, the antipodals of the megagametophytes, residing within an elongate chalazal pan-handle (Fig. 4I), were varied in number: from two (total nuclei within the gametophyte seven) to nine (total nuclei within the gametophyte 14). Eight-, nine-, and ten- nucleate gametophytes (with three, four, and five antipodal nuclei, respectively) accounted for 48 of 63 of the gametophytes (76.2%) inspected.

In terms of reproductive development, *Erigeron* as a whole is notable in exhibiting considerable diversity, unlike the majority of Asteraceae, which possess the classic monosporic *Polygonum*-type development (Harling 1951; Pullaiah 1984). Out of a total of 26 *Erigeron* taxa investigated (Harling 1951), 18 are tetrasporic (69.2%), five are bisporic (19.2%), and only three are monosporic (11.5%). There also appears to be considerable lability in development in the genus; five of the tetrasporic species yield occasional bisporic ovules, two of the bisporic species exhibit occasional monospory and tetraspory. In overall pattern, *E. lemmonii* appears to be most similar to

TABLE 2. SEED FROM CONTROLLED CROSSES FOR *ERIGERON LEMMONII*. Crosses performed for individual capitula selected for the seed parent with pollinations from the pollen parent over a five day period. Percentage cypselae resulting from crosses estimated as number of cypselae divided by the total number of florets (ray plus disc) in the capitulum.

Cross (pollen parent × seed parent)	# Cypselae / # Florets in capitulum	Percentage cypselae formation
1. North Main Face × Boulder	39 / 98	39.8
2. North Main Face × Owl-2	3 / 76	3.9
3. North Main Face × Main Face-2	1 / 119	0.8
4. Owl-2 × Boulder	25 / 85	29.4
Mean (SD)	17.0 / 94.5	11.7 (19.1)

E. glabellus Nutt., which is reported, within one individual, to possess equal proportions of monosporic and bisporic derived megagametophytes. *Erigeron lemmonii* differs in that, in addition to monosporic and bisporic gametophytes, it produces intermediate types and has one megasporocyte per ovule; *E. glabellus* produces 2–11 megasporocytes per ovule (Harling 1951).

Our data from four experimental crosses for *Erigeron lemmonii* show variation in percentage of seed produced per capitulum (0.8 to 39.8%, mean of 11.7 cypselae; Table 2). The North Main Face plant, even when used as a common pollen donor produced only one cypsela (0.8%) when crossed with Main Face-2 but 39 cypselae (39.8%) when crossed with Boulder. These data may indicate reproductive barriers in the population. Given that the *E. lemmonii* population is relatively small and evidently self-incompatible, it is possible that S-allele diversity has been reduced, which would limit opportunities for successful reproduction in the population (Busch and Schoen 2008). Our data at least show that plants are capable of producing seed; the design prevents further strong inference.

Our observations shed light on reproduction in *Erigeron lemmonii*. First, it is surprisingly easy to cultivate vegetatively under standard greenhouse conditions. This means that if the population in Scheelite Canyon were to be threatened, *ex situ* propagation would likely not be problematic. Second, it is diploid ($2n = 18$), sexual, and self-incompatible. This means that it appears to be a distinct evolutionary lineage and is not an asexual polyploid microspecies. This potentially greatly simplifies the rationale and strategy in developing a conservation plan for it (Hey et al. 2003; Ennos et al. 2005). Third, our data show that *E. lemmonii* individuals exhibit a mixture of monosporic and bisporic female gametophyte development. Notwithstanding, this condition does not appear to limit its ability to produce regular appearing mature gametophytes. In the absence of further reproductive and phylogenetic data, it is uncertain if this condition is an isolated occurrence, or if it characterizes a group of related taxa.

ACKNOWLEDGMENTS

We thank Kim A. Whitley, Northern Arizona University, for help collecting ramets, the University of Central Arkansas for the use of greenhouse space, and John Strother (University of California, Berkeley) and an anonymous reviewer for comments that improved the manuscript. Field collection funding was provided by the United States Army's Environmental Quality and Installations Basic Research Program as part of a larger research project (Project 09-03).

LITERATURE CITED

BROUILLET, L., T. K. LOWREY, L. URBATSCH, V. KARAMAN-CASTRO, G. SANCHO, S. WAGSTAFF, AND J. C. SEMPLE. 2009. Astereae. Pp. 449–490 *in* V. A. Funk, A. Susanna, T. F. Stuessy, and R. J. Bayer, (eds.), Systematics, evolution, and biogeography of Compositae. International Association for Plant Taxonomy, University of Vienna, Austria.

BUSCH, J. W. AND D. J. SCHOEN. 2008. The evolution of self-incompatibility when mates are limiting. Trends in Plant Science 13:128–136.

CRONQUIST, A. 1947. Revision of the North American species of *Erigeron,* north of México. Brittonia 6:121–302.

DE NETTANCOURT, D. 1977. Incompatibility in angiosperms. Springer-Verlag, New York, NY.

ENNOS, R. A., G. C. FRENCH, AND P. M. HOLLINGSWORTH. 2005. Conserving taxonomic complexity. Trends in Ecology and Evolution 20:164–168.

GERSTEL, D. U. 1950. Self-incompatibility studies in guayule. II. Inheritance. Genetics 35:482–506.

GORI, D. F., P. L. WARREN, AND L. S. ANDERSON. 1990. Population studies of sensitive plants of the Huachuca, Patagonia, and Atascosa Mountains, Arizona. Unpublished report. Coronado National Forest, Tucson, AZ.

GRAY, A. 1883. Contributions to North American botany. I. Characters of new Compositae, with revisions of certain genera, and critical notes. Proceedings of the American Academy of Arts and Sciences 19:1–96.

HARLING, G. 1951. Embryological studies in the Compositae. Part III. Astereae. Acta Horti Bergiani 16:73–120.

HERR, J. M., JR. 1971. A new clearing-squash technique for the study of ovule development in angiosperms. American Journal of Botany 58:785–790.

HEY, J., R. S. WAPLES, M. L. ARNOLD, R. K. BUTLIN, AND R. G. HARRISON. 2003. Understanding and confronting species uncertainty in biology and

conservation. Trends in Ecology and Evolution 18:597–603.

MALUSA, J. 2006. Surveys and monitoring plots of the Lemmon's fleabane, *Erigeron lemmonii*, in the Huachuca Mts, Arizona. A report submitted to the United States Fish and Wildlife Service, the University of Arizona and the Arizona Department of Agriculture.

NESOM, G. 2006. *Erigeron*. Pp. 256–348 *in* Flora of North America Editorial Committee (eds.), Flora of North America North of México, Vol. 20: Magnoliophyta: Asteridae (in part): Asteraceae, part 2. Oxford University Press, New York, NY.

———. 2008. Classification of subtribe Conyzinae (Asteraceae: Astereae). Lundellia 11:8–38.

NOYES, R. D. 2000. Biogeographical and evolutionary insights on *Erigeron* and allies (Asteraceae) from ITS sequence data. Plant Systematics and Evolution 220:93–114.

——— AND J. R. ALLISON. 2005. Cytology, ovule development and pollen quality in sexual *Erigeron strigosus* (Asteraceae). International Journal of Plant Sciences 166:49–59.

PULLAIAH, T. 1984. Embryology of Compositae. International Bioscience Series 13. Today and Tomorrows Printers & Publishers, New Delhi, India.

STANLEY, R. G. AND H. F. LINSKENS. 1974. Viability tests. Pp. 67–86 *in* R. G. Stanley and H. F. Linskens, (eds.), Pollen: biology, biochemistry, and management. Springer-Verlag, Berlin.

U.S. DEPARTMENT OF THE INTERIOR, FISH AND WILDLIFE SERVICE. 2012. Endangered and threatened wildlife and plants; 12-Month finding for the Lemmon Fleabane, Endangered Status for the Acuna Cactus and the Fickeisen Plains Cactus and Designation of Critical Habtitat: Proposed Rule. Federal Register 77:60509–60579.